II. PROJECT PROCEDURES

A. Formal Speciation and Characterization of the Life Cycle

The systematics of armored dinoflagellates requires knowledge of the arrangement and ornamentation of cellulose deposits called armored plates that are obscured by several layers of membrane coverings on the cell exterior (Bold & Wynne 1985). Only when the membranes are carefully stripped from the cell to clarify apical, ventral, dorsal and posterior views, can the plate arrangement or "formula" be obtained. Following procedures recommended by Dr. K. Steidinger (Florida Department of Environmental Protection - Florida Marine Research Institute, St. Petersburg; pers. comm.), we attempted to remove the outer membranes by preserving dinospores in 2% buffered formalin or acidic Lugol's solution (Vollenweider 1974), followed by immersion in various concentrations of Triton X detergent or ethanol for variable exposure periods.

To describe the life cycle of *Pfiesteria piscimorte* (nov.gen., nov.sp.), we isolated individual toxic dinospores and planozygotes, and viewed them over time (hours) under phase optics with a Olympus BH-2 research microscope or an Olympus IMT inverted microscope at 600x. We observed conversions among all described stages using an Olympus PM-10AD automated photomicrographic camera system. Cultures were also subjected to varying light, temperature, salinity and nutrient enrichment to induce transformations among stages. Video photography enabled us to document some transformations (e.g., from flagellated cells to "star-shaped" filipod amoebae, and then to colorless lobate amoebae).

B. Field Monitoring Effort

Numerous meetings and presentations to regulatory agencies, concerned citizens groups, scientists, the state legislature, and students at the elementary, secondary, high school and college level provided information and garnished support for a "network" of assistance in sampling for the new toxic dinoflagellate during fish kills while in progress. This effort was aided by publication of a suggested sampling protocol in local newsletters such as the *Albemarle-Pamlico Advocate* and the *North Carolina Coastal Federation News* (Fig. 3). In this ongoing effort, we have also designed a poster that will be distributed dockside at fish processing centers to aid in alerting fishermen to the toxic dinoflagellate and obtaining their help in sampling kills they encounter. Further, PI JMB has trained appropriate staff of state regulatory agencies to recognize all stages of the toxic